Oxidative ratio (OR) of southern African soils and vegetation: updating the global OR estimate

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Abstract

Oxidative ratio (OR) is the ratio of moles O_2 released per mole CO_2 sequestered through photosynthesis in the terrestrial biosphere. The lower the OR value the more CO_2 an environment can potentially sequester. It is this property of the organic matter that plays a role in models of CO_2 partitioning between the atmosphere and the biosphere. Recent studies have shown that the accepted value of OR (1.1±0.05) may not be appropriate but that there are a number of research gaps before a full account of global OR values can be carried out.

This study aims to fill some of the research gaps by carrying out a targeted sampling campaign in southern Africa. Vegetation, litter and soil samples were taken from a range of soil orders and biomes across a series of locations in South Africa and Swaziland. From these samples this study has been able to update a recent meta-analysis and show that although there were significant differences between some sites and vegetation types, there was no

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significant difference between the soil orders or biomes sampled. This study has also been able to update the global OR_{terra} estimate to 1.06 ± 0.06.

Keywords

Oxidative ratio; ~~T~~terrestrial carbon cycle; elemental composition; organic matter; Swaziland; South Africa

1. Introduction

Battle et al. (2000) used changes in atmospheric levels of oxygen (O_2) and nitrogen (N_2) to calculate the sizes of annual sinks between global reservoirs and for the carbon dioxide (CO_2) flux to the land proposed the following equation:

$$f_{land} = -\frac{OR_{ff}}{OR_{terra}} f_{fuel} + \frac{1}{(k_1 k_2 OR_{terra})} \frac{d\left(\frac{O_2}{N_2}\right)}{dt} \quad \text{Equation 1}$$

where: f_{fuel} is flux of CO_2 due to fossil fuel combustion; $\frac{d\left(\frac{O_2}{N_2}\right)}{dt}$ is rate of change of the molar ratio of atmospheric O_2 and N_2; OR_{ff} is the combustion stoichiometry; OR_{terra} is the oxidative ratio of the terrestrial biosphere; and $k_1$and k_2 are conversion factors (0.471 and 4.8 respectively).

Within equation (1) the relative partitioning of atmospheric carbon is denoted by the important term – oxidative ratio (OR). The OR is the molar ratio of oxygen (O_2) and carbon dioxide (CO_2) fluxes, associated with either fossil fuel combustion (OR_{ff}) or photosynthesis (OR_{terra}). Given the importance of OR in equation 1 to the estimation of global carbon fluxes research has focussed on the relative changes of atmospheric O_2 and CO_2 (Keeling and Shertz, 1992; Keeling et al., 1996). Until recently there has only been one estimate of OR for

47 the terrestrial biosphere (OR_{terra}) that has been used with equation 1, which came from
48 Severinghaus (1995) who estimated the value to be 1.1±0.05 and this value has been
49 commonly adopted through several global studies (Battle et al., 2000; IPCC, 2007).

50 In a recent meta-analysis of global terrestrial OR values, Worrall et al. (2013) showed
51 that, whilst within the range of natural occurrence, the commonly used value of 1.1 was
52 probably not the most appropriate value. Worrall et al (2013) showed that 1.04 ± 0.03 was a
53 more appropriate choice and that adopting this value meant that the terrestrial carbon sink has
54 been underestimated by up to 14%. However, in their analysis, Worrall et al. (2013) found
55 that there were several soil orders and global biomes (Loveland and Belward, 1997) that were
56 under sampled or for which no OR value existed. They showed that there were only single
57 studies that had sufficient elemental data to calculate OR values for Alfisols, Andisols,
58 Aridosols, Oxisols, and Vertisols and that there were no values for Ultisols. For global
59 biomes there was only one study under woody savannah and no studies with data for
60 shrublands and savannahs; permanent wetlands; or for urban biomes.

61 This study aimed to tackle these some of these knowledge gaps through a targeted
62 sampling campaign in South Africa and Swaziland, an area where a number of the under-
63 sampled soil orders and biomes were located. Furthermore, the review of Worrall et al.
64 (2013) had to re-calculate available data but found no studies which had sampled different
65 carbon pools at the same site, i.e. no study was available that sampled vegetation and its
66 underlying soil, and so therefore it was impossible to judge whether OR was governed by its
67 location, its vegetation or by its soil orders.

68

69 **2. Methods**

70 The approach of this study was to consider the variation in OR across multiple soil orders and
71 multiple biomes. For each site all the possible organic matter pools were sampled with the

view of comparing OR values between sites, organic matter material types and assess variability within sites.

2.1. Field sampling

Sampling was carried out in January 2012 and in total 30 sites were visited across the Gauteng, Mpumalanga and Limpopo provinces of South Africa, as well as in Swaziland (Figure 1; Table 1). At each site, soil, vegetation, and litter were sampled whenever present. This method of compartmentalising the major carbon pools has been found to be a suitable sampling strategy for other OR focussed studies (Clay and Worrall, in press). Soils were sampled from the upper 5 cm using a trowel whilst vegetation was carefully removed using secateurs. Sampling locations were selected so the greatest possible range of USDA soil orders and biomes were visited. For all but Lithosol, each soil order was sampled at two separate locations under distinct biomes. Similarly, each biome was sampled at more than one location; however, it was not possible to perform a complete factorial approach with respect to soil order and biome simply because each possible combination does not exist in nature. Sites were classified into one of 15 biomes based on the IGBP land cover classes (Loveland and Belward, 1997; Loveland et al., 2000) and into one of 12 soil orders of the USDA soil taxonomy. Furthermore, the dominant plant functional type (PFT) was sampled at each site along with any co-dominant PFTs. All samples were bagged in the field and air dried to reduce the moisture content and the possibility of oxidation prior to international shipping.

2.2. Laboratory analysis

Upon arrival in the United Kingdom (UK) all samples were dried at 105°C for 48 hours prior to further analysis. For soils any root matter present was removed and bulk density was then

calculated on a dry weight basis. The soils were pre-treated using a 2% hydrofluoric (HF) acid solution based on the methods of Mathers et al. (2002) and Skjemstad et al. (1994). Approximately 5g of soils were treated with five 50mL aliquots of 2% HF acid and shaken. Supernatants were centrifuged and decanted between treatments. Soils were rinsed with deionised water at least 3 times and then dried at 75°C. In the final stage of sample preparation, the HF-treated soils, along with all litter, and vegetation samples were all ground using a Spex 6770 Cyromill.

For comparative purposes, three standard, naturally-occurring organic biochemical compounds were analysed: lignin, humic acid, and cellulose. The lignin and humic acid were from Aldrich and the cellulose was supplied by Whatman.

Carbon (C), hydrogen (H), nitrogen (N), oxygen (O) (CHNO) analysis

All samples were analysed for CHNO elemental content. Samples were analysed for their carbon, hydrogen and nitrogen concentration on a Costech ECS 4010 Elemental combustion system with pneumatic autosampler. It was set up for CHN analysis where Reactor 1 consisted of chromium (III) oxide/Silvered cobaltous-cobaltic oxide catalysts at 950°C and Reactor 2 consisted of reduced high purity copper wires at 650°C. Helium was used as the carrier gas at a flow rate of 95 ml min^{-1} and oxygen at a flow rate of ~30 ml min^{-1}. This was filtered for hydrocarbons upstream of the instrument. A packed 3m gas chromatograph (GC) column was used for separation of the gases. A thermal conductivity detector (TCD) was used to calculate the signal of each sample. For oxygen concentration, the Costech ECS was also used but was set up for O analysis. Reactor 1 consisted of a nickelised carbon/silica chips/nickel wool pyrolysis tube at 1060°C whilst Reactor 2 was left empty. Helium was used as the carrier gas at a flow rate of 130 ml min^{-1} but no oxygen was used. A 2m packed oxygen GC column was used for separation of the gases. Chloropentane vapour was added to

122 the carrier gas to enhance decomposition of the oxygen compounds and to reduce possible
123 memory effects from previous samples (Kirsten, 1977).

124 The computer software used was EAS Clarity (DataApex Ltd, Prague, Czech
125 Republic). For both CHN and O setups calibration curves of $r^2 > 0.999$ were created using
126 acetanilide as the standard. Samples of acetanilide were included within each run as unknown
127 samples to act as internal quality control checks. Each sample (litter, soil, or vegetation) was
128 analysed in triplicate (i.e. three times in one run on the CHN setup and a further three times in
129 one run on O set up), and a mean value calculated for C, H, N and O for each sample

130

131 *Calorimetry*

132 Energy content, as gross heat value (ΔH_c), was measured for all vegetation and litter samples.
133 Even after HF treatment the mineral content of the soil samples was still too high for an
134 energy content to be measured. Masiello et al. (2008) has shown that it is possible to derive
135 carbon oxidation state (C_{ox}) values (and therefore OR values) from calorimetry data. Analysis
136 was performed on a 6200 Isoperibol Calorimeter (0.1% Precision Classification, Parr
137 Instrument Company, Illinois, USA) with 1108(P) Oxygen Bomb. Calibration was performed
138 as a rolling average of 10 measurements using benzoic acid standards. Samples were placed
139 in crucibles and compressed to stabilise the surface and weighed following compression, with
140 a weight of approximately 0.8 g used. Where sample amount was deficient, a benzoic acid
141 spike was used. Following analysis, fuse corrections were performed by measuring the length
142 of fuse wire remaining, measured in calories, and converting to kJ/g using a constant. The
143 correction value was subtracted from the energy content recorded during analysis.

144 Masiello et al. (2008) used ΔH_c from calorimetry to calculate C_{ox} values for a range of
145 standard materials. If there is a relationship between C_{ox} and ΔH_c, it might be reasonable to
146 expect there to be a relationship between ΔH_c (measured using calorimetry) and OR values

147 (calculated from elemental analysis) from this study. Clay and Worrall (in press) were able to
148 plot ΔH_c and OR values to identify unusual observations that may explain the variation in OR
149 between sites in a study of OR variation across UK peatlands. Therefore, ΔH_c values were
150 plotted against OR values for the vegetation and litter along with the standard materials –
151 cellulose, lignin and humic acid. Only vegetation and litter samples were included in this
152 analysis.
153
154 *C_{ox} and oxidative ratio (OR) calculation*
155 A value of OR can be calculated from a carbon oxidation state, which in turn can be
156 calculated from elemental compositions of organic matter as follows (Masiello et al., 2008):
157
158 $$C_{OX} = \frac{2[O]-[H]+3[N]}{[C]}$$ Equation 2
159
160 Where: [X] = molar concentration of C, H, N or O; and assuming the majority of organic
161 nitrogen exists as amine groups in amino acids. Furthermore, sulphur is not included in this
162 equation as it is assumed to form < 0.25% of biomass (Charlson et al., 2000).
163 As C_{ox} and OR are related through the balancing of organic matter synthesis, the OR
164 value is calculated as the ratio of O_2 and CO_2 coefficients (for further details see Masiello et
165 al. (2008)). Simplified it is then calculated as:
166
167 $$OR = 1 - \frac{C_{ox}}{4} + \frac{3[N]}{4[C]}$$ Equation 3
168
169 Equation 3 assumes that there is no contribution to the C_{ox} from S or P, and it has been shown
170 that the error from this assumption would be only ± 0.002 (Hockaday et al., 2009). This

equation also assumes that the nitrogen source in carbon fixation is N_2. There are two further possible nitrogen conversions (Masiello et al., 2008):

Ammonia (NH_3): $$OR = 1 - \frac{C_{OX}}{4}$$ Equation 4

Nitrate (HNO_3): $$OR = 1 - \frac{C_{OX}}{4} + \frac{2[N]}{[C]}$$ Equation 5

For the purposes of this paper, Equation 3 was used as N_2 is the dominant form in the ecosystem. The agricultural soils will likely have received N in other forms in addition to N_2, but no fertilizer data were available for these sites and other studies have shown minimal changes in OR when using alternative assumptions for the reason that [N]/[C] is always likely to be < 0.1 (Gallagher et al., 2014).

As a quality control check, OR values were only calculated for those samples that had measured data for C, H, N and O; if one of these data were missing (e.g. lost sample), no OR value was calculated.

2.3. Statistical analysis

The design of the study allows for several statistical comparisons to be made using an ANOVA approach. Firstly, one-way ANOVA was used to test whether there were significant differences in OR between the organic matter material types being considered, i.e. soil vs vegetation vs litter. Secondly, this set of ANOVA could be sub-divided into separate ANOVA: the difference in OR between soil orders; the difference between biomes; the difference in vegetation functional groups.

Finally, the variation in organic matter material types (soil, vegetation and litter) between sites was examined. In this final ANOVA, it was possible to analyse the differences between soils, vegetation and litter across the sites considered in study for the element ratios

196 and OR. However it was not possible to include energy content as no values were measured
197 for soils. Furthermore, as litter was not collected on all sites it was not possible to compare all
198 sites directly, with respect to organic matter material type. Sites without litter were removed
199 from the ANOVA and only those sites with soil, vegetation, and litter were included in
200 further analysis (n = 14).

201 The response variables (C/N ratio, H/C ratio, O/C ratio, energy content, Cox, and OR)
202 were tested for normality prior to ANOVA using the Anderson-Darling test. If the response
203 variable failed the test it was log-transformed and re-tested – it did not prove necessary to
204 further transform the data. Post-hoc testing of the results was performed using the Tukey test
205 at 95% level to determine significant differences between levels of any factor. The
206 magnitude of the effects of each significant factor and interaction were calculated using the
207 generalized ω2 (Olejnik and Algina, 2003). All results are reported to a significance level of
208 $p<0.05$.

209

210 *2.3.1. Global OR values*

211 The data from this survey is used to update the estimation of global OR made by Worrall et
212 al. (2013). Worrall et al. (2013) used a weighted average based upon the residence time of
213 carbon in the vegetation and soil carbon pools as this better reflected the faster turnover of
214 carbon in the vegetation pool compared to the soil carbon pool. Therefore, OR_{terra}^{global} is:

215

216 $$OR_{terra}^{global} = f_{soil}^{terra} OR_{soil}^{global} + f_{veg}^{terra} OR_{veg}^{global} \quad \text{Equation 6}$$

217

218 Where: OR_{terra}^{global} = the oxidative ratio of the global terrestrial biosphere; OR_{soil}^{global} = the
219 oxidative ratio of global soils; OR_{veg}^{global} = the oxidative ratio of global vegetation; f_{soil}^{terra} =

the proportion of the terrestrial biosphere C annual flux that is due to soils; and f_{veg}^{terra} = the proportion of the terrestrial biosphere C annual flux that is due to vegetation.

The proportion of annual global flux from the soils or vegetation (f_{soil}^{terra} and f_{veg}^{terra} respectively) were based upon the size of each carbon pool divided by the average residence time of the carbon in that pool:

$$1 = f_{soil}^{terra} + f_{veg}^{terra} \quad \text{Equation 7}$$

$$f_{soil}^{terra} = \frac{\varphi_{soil}^{terra}}{t_{soil}^{terra}\left(\frac{\varphi_{soil}^{terra}}{t_{soil}^{terra}} + \frac{\varphi_{veg}^{terra}}{t_{veg}^{terra}}\right)} \quad \text{Equation 8}$$

Where: φ_x^{terra} = the proportion of the terrestrial carbon pool that is in x, with x either soil or vegetation; and t_x^{terra} = average residence time of carbon in the terrestrial carbon pool represented by x, with x as either soil or vegetation (years). The comparative sizes of the soil and vegetation carbon pools were estimated from Eswaran et al. (1993) and Olson et al. (2001) where the proportion of carbon in the vegetation pool was 0.28 and in the soil pool as 0.72. The average carbon residence time for soils was taken as between 20 and 40 years based upon a study by Jenkinson and Rayner (1977). The average carbon residence time for vegetation was taken as between 2 and 5 years (e.g. Gaudinski et al., 2000). Given the above approach the values of f_{soil}^{terra}= 0.27 and f_{veg}^{terra}= 0.73.

The value of OR_{veg}^{global} was calculated as the weighted average of the expected value of OR for each of 15 global biomes (Loveland and Belward, 1997) where the weighting factor was the area of each biome:

$$OR_{veg}^{terra} = \frac{1}{A_{total}} \sum_{1}^{15} A_n E(OR_n) \quad \text{Equation 9}$$

Where: A_n = the area of biome n (km^2); A_{total} = the total area of all n biomes (km^2); and $E(OR_n)$ = the expected value of the OR of biome n. Given the lack of data for most biomes the median was taken as the expected value.

Similarly, OR_{soil}^{global} was calculated as a weighted average of the expected values of the OR for each of the 12 USDA soil taxonomy soil orders (although Gelisols are treated as equivalent to Histosols) where the weighting factor was the total carbon content of each soil order as estimated by Eswaran et al. (1993) – note that such estimates of carbon content are not available for separate global biomes.

$$OR_{veg}^{terra} = \frac{1}{OC_{total}} \sum_{1}^{11} OC_n E(OR_n) \quad \text{Equation 10}$$

Where: OC_n = the organic carbon content of soil order n (Pg C); OC_{total} = the total organic carbon content of all n soil orders (Pg C); and $E(OR_n)$ = the expected value of the OR of soil order n. As above, given the lack of data for most soil orders, the median was taken as the expected value.

3. Results

In total 42 vegetation samples, 14 litter samples and 30 soil samples were analysed. Table 2 shows the elemental concentration data, energy content, C_{ox} and OR values for each of the material types whilst Tables 3, 4 and 5 show the data for each of the soil orders, biomes and main vegetation types respectively. None of the datasets needed to be transformed prior to ANOVA. Of the three material types, soils had the lowest OR values (highest C_{ox} values, i.e. the most oxidised) compared to vegetation or litter, which appear to have very similar values

of OR. This study has chosen to focus upon what might, *a priori*, be considered as highly oxidised soil orders, especially Ultisols and Oxisols, and so the relative OR of soils and vegetation and litter may reflect this highly oxidised state by having comparatively similar values in contrast to other soil orders~~values closer to …~~. Median OR values for material types are all lower than 1.1 though still within the range of results reported by Worrall et al. (2013).

3.1. Variation within organic matter material type

Soil orders

The one-way ANOVA of the soils data (Supplementary Table 1) showed no significant differences in elemental ratios, C_{ox}, or OR between soil orders.

Biomes

A one-way ANOVA of the data divided into biomes (Supplementary Table 2) showed no significant differences in elemental ratio, ΔH_c, C_{ox} or OR.

Vegetation functional groups

There were significant differences between vegetation functional groups in the elemental ratios. For C/N ratio, the significant difference ($p = 0.003$) lay between tree branches and all other vegetation types, with the exception of sugar cane samples. The highest C/N ratios were found in tree branch samples (Supplementary Table 3). The O/C ratio showed a significant differences ($p = 0.005$) where the difference lay between tree leaves and crops, grass and tree branches. In this instance the lowest O/C ratios were found in tree leaves whilst the higher values of O/C were found in crop, grass and tree branch (Supplementary Table 3). For H/C ratios the only significant difference ($p = 0.005$) was found between tree leaves and grass.

There were no significant differences in the ANOVA models for ΔH_c ($p = 0.668$), C_{ox} ($p = 0.100$) or OR ($p = 0.053$). However, within the post-hoc testing for OR there were significant differences between tree branches and leaves, no matter where the leaves came from. In this case the OR of the tree branches was significantly lower than that of the leaves (Supplementary Table 3).

3.2. Organic matter material type vs. site

Site was a significant factor for both O/C and H/C ratios (Table 3) explaining 31% and 17% of the variation in the data respectively. Post-hoc testing showed that the highest ratios were found on sites 18, 19 and 21 which were those sites under sugar cane plantations (Table 1). Specifically for O/C ratios, site 18 was different from sites 3, 7, 10, 26, and 28 whilst site 19 was different from site 7. The sites 3, 7, 10, 26, and 28 are predominately those with trees or 'woody' stems (Table 1). For H/C ratios, site 18 was different from sites 3, 5, 7, 11, 26, 28, and 29 whereas site 19 was different from 3, 5, 7, 11, 12, 26, 27, 28, and 29 whilst site 21 was different from site 7. Again the differences appear to be between sugar cane plantations and those with the presence of trees. There were no significant differences ($p > 0.05$) between the OR, or C_{ox} of sites.

Material type was a significant factor in the case of H/C ratio, C_{ox} and OR explaining 36%, 42% and 30% of the variation in the data respectively (Table 3). Post-hoc testing showed ~~than~~ that in the case of H/C ratio soil samples had a lower value than for either vegetation or litter. For C_{ox}, soil had a higher value than either vegetation or litter, whilst OR had the opposite pattern with soil having a lower OR value than either vegetation or soil. This echoes the general pattern in material types seen in Table 2.

There were significant interactions between site and material types for O/C and H/C ratios explaining 47% and 40% of the variation in the datasets respectively (Table 3). In the

O/C data soils generally had lower or very similar values to vegetation and litter but for sites 18, 19 and 21 there is a pronounced switch with these sites having higher soil OR values than either vegetation and litter. The pattern was similar for the H/C ratio where soil had lower H/C values than litter and vegetation but on sites 18 and 19 soil OR values were greater than litter or vegetation. No significant interactions were found for C/N ratio, C_{ox} or OR.

3.3. Variation in organic matter composition

Masiello et al. (2008) showed a positive correlation between OR and ΔH_c, and indeed, for the data from this study a correlation can be observed between the OR and ΔH_c of the organic material standards (humic acid, cellulose and lignin; Figure 2; note this correlation is for the standards only and that none exist for the samples). All the vegetation and litter samples plot on or above this line with lower ΔH_c values than would be expected for their OR values. Furthermore, all samples except one appear to lie between three end-members where the end-members are lignin, humic acid and a third end-member of unknown composition (Labelled 'A' on Figure 2). This third end-member lies at a low ΔH_c value whilst having an OR value of approximately 1.11 and similar to lignin. Clay and Worrall (in review) have also identified a similar end-member (high OR with a relatively low ΔH_c) in their assessment of OR values in UK peats. It is possible that soil samples had had an input of mineral matter such as silt effectively reducing the energy value whilst retaining an overall OR signal – OR is a ratio and so not diluted by the presence of mineral matter which is removed through HF digestion. However, in this mixing diagram, only vegetation and litter were considered. Instead the end-member may be a specific compound in certain samples that is in a reduced form but has a low calorific value. For reference, nearby litter and vegetation samples came from a mixed forest and grasslands, respectively.

345 3.4. Global OR

346 In Worrall et al. (2013) the value of OR_{veg}^{global} was based on an analysis of 32 samples, this can
347 now be updated to include 213 samples (Table 4) and the biome area weighted value of
348 OR_{veg}^{global} = 1.07 ± 0.02, where the uncertainty is the inter-quartile range. Similarly, OR_{soil}^{global}
349 was based upon 33 samples and this can now be updated to include analysis of 490 samples
350 (Table 5) and gives an organic carbon content weighted value of OR_{soil}^{global} = 1.06 ± 0.04.
351 Given the updated values, the residence time weighted value of OR_{terra}^{global} (Equation 6) can
352 now be estimated as 1.06 ± 0.06. By applying this new value of OR_{terra}^{global} to equation (1), the
353 new value of f_{land} will be 1.45 Gt C/yr.

354

355 **4. Discussion**

356 This study was specifically designed to fill in some of the data gaps identified from Worrall
357 et al. (2013) and as such is able to present OR data for the first time for Ultisols and to
358 expand the database of OR values for Alfisols, Entisols, Histosols, Oxisols and Vertisols.
359 This work is also able to report, for the first time, OR values for savannahs and to increase
360 the database for woody savannahs, evergreen, deciduous, and mixed forest, and grasslands.
361 Furthermore, recent additional studies (Clay and Worrall, in press; Worrall et al., in review)
362 have also presented OR data from a range of soil and vegetation types since the original
363 meta-analysis.

364 This study has shown that it is possible to distinguish between major carbon pools i.e.
365 between vegetation and soils or between litter and soil, but that vegetation and litter are not
366 significantly different from each other. Vegetation and plant litter can be described as lying
367 on a decay continuum (Melillo et al., 1989; Fang et al., 2011) and as such it may be that the
368 samples chosen are closer on this continuum ~~that~~ than they are to the underlying mineral soil.
369 In this study, the soils were found to have significantly (p = 0.001) lower OR values (Median

370 = 1.02) than vegetation (Median = 1.07) which is the reverse of that found in Worrall et al.
371 (2013) where vegetation OR was generally lower than soil OR. However, that previous study
372 could not consider, nor had very few samples of, the soil orders sampled here, in particular
373 for Oxisols and Ultisols. Oxisols and Ultisols are typified by old and oxidised soils compared
374 to the other soil orders and therefore might be considered to have highly oxidised organic
375 content. It should also be noted that this study found higher values of vegetation OR than
376 previously reported, and for all the global biomes that could be considered in this work, for
377 which a previous estimated was made, this study found higher values of OR.

378 The significant difference between soil and vegetation pools justifies the method of
379 Worrall et al. (2013) as expressed in Equation 6. However, this approach was based on the
380 idea that OR_{veg}^{global} and OR_{soil}^{global} could be understood from the combination of results for a
381 range of global biomes and soil orders. This was partly out of necessity as it was only
382 possible to classify results from disparate literature studies into broad, globally-applicable
383 classes. However, this study found no significant difference between soil orders. Clay and
384 Worrall (in review) did find a significant difference between Histosols and Inceptisols from
385 eight locations across the UK, while the present study could find no difference between any
386 soil orders. It may therefore be that not all soil orders are different and that the biggest
387 difference is between organic-rich and mineral-rich soils. Similarly this study could not find a
388 significant difference between the biomes.

389 There may not have been significant differences between the sampled soil orders and
390 biomes but there were significant differences between vegetation functional groups with
391 respect to elemental composition and possibly OR. This perhaps suggests that the control on
392 differences in OR are due to varying proportions of biochemical compounds between
393 vegetation, sites or biomes. For example, the comparison of OR and ΔH_c (Figure 2) shows
394 that variation in the OR of vegetation and litter samples is bracketed by the reduced lignin

and the comparatively oxidised cellulose and so variation in these plant components could control the OR of the biome. This study was careful to be the first to sample all major organic matter material types wherever available on any site but it did not quantitatively recover the biomass and so variation between biomes may just reflect the choice of sample within a biome rather than the whole biome. Additionally, this study did not look at the role of roots in with respect to OR. Roots represent a large global carbon store (~268Pg, Robinson, 2007) and given their significant role in soil respiration (Hanson et al., 2000; Bond-Lamberty et al., 2004), it may be that for complete ecosystem OR estimates, the OR of roots will need to be calculated. Therefore, there is a need in the future to quantitatively recover and sample the biomass within a biome. The difference found between sites within this study may well reflect this difference between biochemical compounds and hence the difference between sugar cane plantations and sites with woody vegetation.

By assessing the OR of each carbon pool at a sampling site, this study is able comment on what kind of sample may be representative of the OR of the environment. In terms of O/C and H/C ratios there was a significant interaction between site and material types indicating that organic matter material types varied amongst site. This might imply that classification by material types is site-dependent and not suitable for large-scale surveys. However this was only seen for two ratios and the lack of an interaction between site and material type for OR suggests that the difference between organic matter material types is independent of the change between sites and as such it is appropriate to sample all the major carbon pools in OR assessments.

This study, along with other studies, can now dramatically increase the amount and diversity of data that can be used to calculate the global OR estimate – - OR_{terra}^{global}. It is interesting to note that values have converged and not diverged, with OR_{soil}^{global} and OR_{veg}^{global} no longer significantly different from each other. There is still a large variation in the density

of sampling of OR of both biomes and soil orders, but given the findings of this study there are key contrasts that should now be focused on. For example, there is still only one study each of Aridisols and Andisols but it may be more important to consider contrasts between organic-rich and organic-poor sub-orders rather than between the orders themselves. Equally, the contrast in biomes may be particularly pronounced during land use change and given the results here we suggest that the contrast between forest and grassland might be particularly large.

5. Conclusions

This study showed that there were significant variations in oxidative ratio (OR) between organic matter material types and that OR values were lowest in the soils relative to vegetation and litter. The analysis suggests that OR variation may be controlled by varying proportions of organic matter biochemical compounds such as lignin and cellulose. This study has also been able to add new data to the expanding literature on OR and can update the global OR_{terra} estimate to 1.06 ± 0.06.

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520 Figure 1. Location map of sampling sites in South Africa and Swaziland

521

522 Figure 2. Plot of OR and ΔHc values for vegetation and litter. Standard materials (cellulose,
523 lignin and humic acid) are included for comparative purposes. Linear trend line fitted to
524 standard materials (cellulose, lignin, humic acid). For the purposes of the mixing diagram,
525 humic acid and cellulose form two end-members, whilst the approximate position of the third
526 end-member, labelled A, is of unknown composition.

527

528 Table 1. Site location with major descriptive information and samples collected.

529

530 Table 2. Median values (inter-quartile range in parentheses) for each measured or derived
531 variable for the three material types.

532

533 Table 3. ANOVA for site and material types for elemental ratios, C_{ox} and OR values. df =
534 degrees of freedom, p = probability of factor being zero, ω^2 = generalized proportion of
535 variance explained.

536

537 Table 4. The range and median values of OR for each of the global biomes. Additional data
538 from [1] Worrall et al. (in review), and [2] Clay and Worrall (in review).

539

540 Table 5. The range and median values of OR for each of the USDA global soil orders,
541 (Gelisols included with Histosols). Additional data from [1] Worrall et al. (in review), and [2]
542 Clay and Worrall (in review).

543

544

545 Supplementary Table 1. Median values (inter-quartile range in parentheses, where applicable)
546 for each measured or derived variable for soil orders.

547 Supplementary Table 2. Median values (inter-quartile range in parentheses, where applicable)
548 for each measured or derived variable for biomes.

549 Supplementary Table 3. Median values (inter-quartile range in parentheses, where applicable)
550 for each measured or derived variable for the main vegetation functional groups

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